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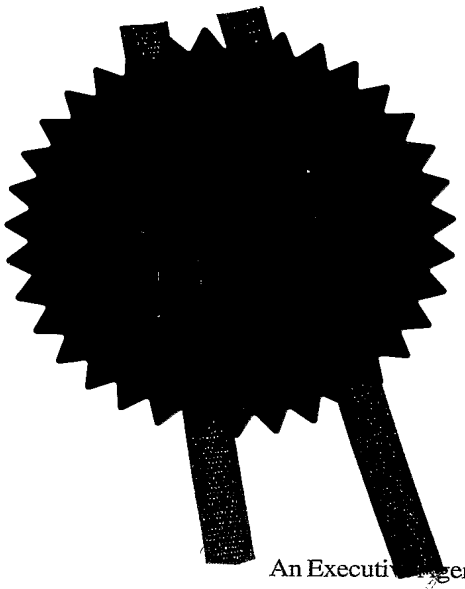

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1. Your reference — SJC/P/14421.4GB

2. Patent application number
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3. Full name, address and postcode of the applicant or of each applicant *(underline all surnames)*

City University, London
Northampton Square
London EC1V 0HB
United Kingdom

Patents ADP number *(if you know it)*
If the applicant is a corporate body, give the country/state of its incorporation — 8772253001

4. **Title of the invention** — CHARACTERISING BODY TISSUE

5. Name of your agent *(if you have one)* — Olswang

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Country	Priority Application Number *(if you know it)*	Date of Filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

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Drawings*(s)*	6 +6 SD

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Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

Signature OLSWANG Date 23 April 2004

12. Name and daytime telephone number of person to contact in the United Kingdom Stephen Carter 0207 067 3278

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Characterising Body Tissues

Field of the Invention

The present invention relates to methods for the characterisation of body tissue. More specifically, the invention is concerned with the characterisation of body tissue as normal (e.g. healthy) or abnormal (e.g. pathological). The invention has particular, although not necessarily exclusive, applicability to the diagnosis and management of cancer, including breast cancer.

Background

In order to manage suspected or overt breast cancer, tissue is removed from the patient in the form of a biopsy specimen and subjected to expert analysis by a histopathologist. This information leads to the disease management program for that patient. The analysis requires careful preparation of tissue samples that are then analysed by microscopy for prognostic parameters such as tumour size, type and grade. An important parameter in tissue classification is quantifying the constituent components present in the sample. Interpretation of the histology requires expertise that can only be learnt over many years based on a qualitative analysis of the tissue sample, which is a process prone to intra observer variability.

Despite the relative value of histopathological analysis, there remains a degree of imprecision in predicting tumour behaviour in the individual case. Additional techniques have the potential to fine-tune tissue characterisation to a greater degree than that currently used and hence will improve the targeted management of patients.

There remains in particular a need for techniques that can be used to characterise tissue to a greater degree *in vivo*, in order that the need for often painful and distressing biopsies can be reduced.

Summary of the Invention

In general terms the present invention is based on a recognition that Compton scattering densitometry techniques can be used in the analysis of body tissue to very effectively discriminate healthy and abnormal or diseased tissue and to discriminate types of abnormal tissue. Moreover, Compton scattering has been recognised as having potential for application to *in vivo* tissue characterisation techniques.

The invention provides a method for analysing and / or characterising body tissue, the method comprising:

obtaining Compton scatter data measured from a body tissue sample on which a penetrating (e.g. X-ray) radiation beam is incident; and

using the data to provide an analysis and/or characterisation of the tissue sample.

Compton scatter results from an interaction that occurs between a photon and an electron. For this interaction the electron is assumed to be unbound and acting as a free particle. This assumption can be made if the energy of the incident photon is much greater then the binding energy of the atom. Figure 1 illustrates the Compton interaction, where E_0 is the energy of the incident photon, E_1 is the energy of the scattered photon, m_0c^2 is the rest mass energy of the electron and θ is the scattering angle of the photon and φ is the scattering angle of the electron. T is the kinetic energy imparted to the electron.

The electron taking part in the interaction is assumed to be stationary, i.e. the initial energy (E_e) and momentum of the electron equals zero. During the interaction the photon imparts some of its energy to the electron. The amount of energy transferred determines the angle of the recoil of the electron and the angle of the resultant photon.

The angle and energy of a Compton scattered particle can be accurately calculated using the principle of conservation of energy and momentum. From Figure 1 it can be seen that the incident photon has energy $E_0 = h\nu$ and the

scattered photon has energy $E_1 = h\nu'$. Resolving the energy and momentum into parallel and perpendicular components gives the important Compton scatter equation

$$E_1 = \frac{E_0}{1 + \left(\frac{E_0}{m_0 c^2}\right)(1 - \cos\theta)}$$

hence a measure of Compton scatter can be made by detecting the appropriate energy photons at a given angle.

In some instances it may be sufficient for the Compton scatter data to be as simple as a count of photons detected at a selected angle/energy in a given time period. In other instances, it may be desirable to obtain an absolute measure of electron density (or some other derived measurement). Particularly in the latter case, the Compton scatter data is preferably corrected for attenuation in the tissue sample.

One way to compensate for attenuation effects is to use two radiation sources and two detectors. This is an approach commonly used in bone densitometry, but is less preferable when examining tissue samples, particularly *in vivo*, because it results in a greater dose of radiation.

A preferred method to correct for attenuation effects is to obtain data representing a measure of the directly transmitted x-ray radiation for each Compton scatter measurement. This data can then be used to correct the Compton scatter data for attenuation in the tissue sample.

Especially at low angles (less than 90°), it is also important to be able to distinguish Compton scatter measurement from the coherent scatter peak. So, where the transmitted radiation is to be used to correct for attenuation it is preferable that the energy of the scattered photons detected is as close as possible to that of the transmitted radiation. This ensures that the attenuation coefficients are not too different for the two measurements. The energy of the incident penetrating radiation beam and the angle selected for Compton

scatter measurement are chosen such that the Compton and coherent scatter peaks can be resolved, whilst minimising the separation (i.e. energy) of these peaks. This substantially eliminates self-attenuation effects as it allows one to assume that the attenuation coefficients in the sample affecting both peaks are substantially the same.

Preferably the data is used as the input to a predefined calibration model that relates the Compton scatter data to one or more tissue characteristics (e.g. normal or abnormal). It is particularly preferred that the Compton scatter data is used as an input to a multivariate model as described in our co-pending UK patent application GB0328870.1

Brief Description of the Drawings

An embodiment of the invention is described below by way of example with reference to the accompanying drawings, in which:

Figure 1 illustrates the energetics of Compton scattering;

Figure 2 shows schematically the experimental set-up used for Compton scatter measurements in an example of an embodiment of the invention;

Figure 3 illustrates the sample holder used in the Compton scatter measurement of the example;

Figure 4 shows the peak measured with the Ortec GLP-25300 HPGe detector, used in the experiment, using an Am-241 source;

Figure 5 is a schematic of the electronics used for electron density measurements;

Figure 6 shows an observed scatter spectrum obtained for one sample during the experiment;

Figure 7 shows the apparatus of Figure 2 set-up to take transmission measurements;

Figure 8 shows a calibration graph for the electron density measurements;

Figure 9 is a graph of differential scatter coefficient versus theoretical electron density;

Figure 10 shows the results from the Compton scatter measurements taken from all samples during the experiment;

Figure 11 is a graph of tabulated tissue values and experimental data.

Description of Embodiments

The invention is exemplified below with reference to *in vitro* Compton scatter measurements from uniform samples of body tissue. The general technique is, however, equally applicable to the analysis of non-uniform tissue samples, including *in vivo* applications.

The experiment was undertaken in two sections; Compton scatter measurements were made on all the samples, followed by transmission measurements. This was done in preference to the two measurements being made consecutively for each sample. This method was adopted for two reasons; firstly to ensure consistency of set-up between samples through minimising the moving of equipment and secondly to save time.

Compton scatter measurements

Method

Ideally a monoenergetic source should be used to ensure that the Compton scatter peak is easily detectable. The characteristic lines produced by the x-ray tube were used to generate a pseudo-monoenergetic source. Using this method the Compton and Coherent peaks can be easily resolved and windowed. The bremsstrahlung background can then be subtracted.

The desired outcome from the experiment was to be able to resolve the Compton and coherent peaks, whilst keeping them as close in energy as

possible. The detector characteristics dictate that the minimum resolvable energy is about 1 keV.

Using the Compton scatter equation set out further above the angle required to give an appropriate difference in energy between the incident and scattered peaks was calculated. To obtain $E_0 - E' \cong 1$ keV, there was a choice between either using a higher energy and a small scattering angle (defined as θ in Figure 2 below) or a lower energy with a larger scattering angle. Both of these options were explored. The conclusion was that using a higher energy and minimising the angle offered a number of advantages. Firstly the attenuation of the beam by tissue will be lower with a higher energy. Secondly the scatter is at a maximum in the forward direction and at a minimum at 90°. Therefore the flux reaching the detector will be much higher with a smaller angle, reducing count times considerably. A smaller beam size can also be used, improving the accuracy of the measurement. Larger scattering angles can, however, be used if desired.

The distance between the source, sample and detector were kept to a minimum to decrease the loss of flux due to inverse square law effects. The experimental set-up is shown in figure 2.

The incoming x-ray beam was collimated to a 0.5mm circle, both before and after the sample. This was the smallest beam size obtainable whilst maintaining a reasonable flux. The K_α lines from the tungsten target of the x-ray source ($E_{k\alpha 1}$=59.3keV and $E_{k\alpha 2}$=57.97 keV) were used. At this energy a scattering angle of 30° would give a peak separation of 1 keV between the Compton and coherent peaks. The exact angle that was set-up in this example was 28.2°. The scattering volume comprises the tissue contained within the intersecting area of the incoming and scattered beam. For this beam collimation and a scattering angle of 28.2° the entire scattering volume was contained within the sample. This means that no air or plastic was contained within the scattering volume. The samples were measured for 20 minutes per position for 12 positions around the sample.

Samples

5 samples of each tissue type were chosen for examination. These were 5 fibroadenoma (benign), 5 invasive ductal carcinomas (malignant) and 5 pure adipose (normal) samples. The samples were placed into plastic pots of 6mm inside diameter and 1mm wall thickness (illustrated in Fig. 3). Although the walls of the container were relatively thick and would cause significant attenuation of the scattered beam, these containers were chosen because they offered a number of important advantages:

1) The sides were completely rigid so the samples could be placed into the pots and lightly compressed with a stopper (see Figure 3) to remove any air gaps without the pot becoming distorted. This also minimises tissue movement throughout the experiment.
2) The pots were cheap so each sample could have its own pot for the duration of the experiment, making it possible to move the sample and reposition it accurately
3) The samples also needed to be symmetrical about a centre of rotation.

Equipment

Detector

The experiments were performed in the City University Radiation Laboratory using a Pantak HF160 industrial x-ray tube. An HPGe detector was used. This was because a good energy resolution was required for this experiment, to enable the resolution of the Compton and coherent peaks. A peak measured with the Ortec GLP-25300 HPGe detector, which was the detector used throughout all experiments, using an Am-241 source is shown in Figure 4. The energy resolution is calculated as the FWHM of the peak, as illustrated in figure 4. For this detector the energy resolution is 0.435 keV at 59.54 keV (0.73%). The resolution for a NaI(Li) detector at the same energy is about 6-

7%. The reason this peak was used to find the energy resolution is because the experiments that were carried out were done using the 57.97keV $K_{\alpha 2}$ peak from tungsten. This is very close in energy to the Am-241 peak at 59.54 keV and so the resolution will be approximately the same.

Electronics

A diagram of the electronics chain is shown in Figure 5.

The detector was connected via a pre-amp and an amplifier to two single channel analysers, one to record the Compton peak and one to record a background region. Communication with a PC was enabled via an Ethernet card.

Windowing

An observed scatter spectrum is shown in Figure 6. The two coherent peaks of the $K_{\alpha 1}$ and $K_{\alpha 2}$ W lines can be identified. The two, smaller Compton scatter peaks can be seen.

The $K_{\alpha 2}$ Compton peak was measured for this experiment. This is because the $K_{\alpha 1}$ peak, although it has a stronger signal, is significantly overlapped by the two coherent peaks.

Transmission measurements

Method

The transmission measurements are a measure of the reduction in intensity of the unscattered peak and are a measure of the loss of counts due to tissue attenuation. For these measurements the detector was placed at zero degrees (See Figure 7).

System calibration

As the composition of the tissues being measured is unknown, the electron density measurement system needed to be calibrated. This was done by measuring some substances with a known or calculatable electron density. 5 substances were chosen in order to produce a comprehensive calibration graph. The solutions chosen were water, iso-propanol, and solutions of potassium hydrogen phosphate K_2HPO_4. Water and propanol were chosen because they are readily available, easy to handle and have a known electron density that is close to that of tissue. K_2HPO_4 was chosen because it contains elements similar to those found in cellular fluids and so is a good model for human tissue composition. The concentration of the phosphate solutions could easily be varied to provide solutions with differing electron densities. In order to have values close to that of tissue, solutions of 2%, 5% and 10% were used.

In order to verify the scatter data for the calibration solutions the linear differential scattering coefficient can be calculated theoretically as the composition of these solutions is known.

The Klein-Nishina cross-section is dependent on incident photon energy and scattering angle. The Klein-Nishina differential scattering cross section is calculated to be 7.177×10^{-26} cm^2/electron for 57.97 keV photons at a 28.2° scattering angle. Using this value and tabulated values for S(x) taken from Hubbell et al (1975) a value for $\mu_{Compton}$ for each calibration solution was calculated.

A graph showing the experimental values against the theoretical values is shown in Figure 8. The corrected scatter counts are the counts measured in the scatter peak corrected for attenuation and are given by

$$S_{corr} = \frac{[S_{meas} - B_s]}{\left[\left(\frac{I_{meas} - B_T}{I_0 - B_0}\right)\right]} \qquad A(4.13)$$

where S_{corr} is the counts recorded in the scatter peak corrected for attenuation. S_{meas} is the number of counts in the scatter peak, B_s is the background counts in the scatter peak, I_{meas} is the number of counts in the transmitted peak, B_T is the number of background counts in the transmitted peak, I_0 is the unattenuated count intenstiy and B_0 is the background area for these counts.

The graph of Figure 8 can be used to convert the corrected counts measured into differential scatter coefficients for Compton scatter, μ_s, where

$$\mu_s = k[S_{corr}] + N \qquad A(4.14)$$

In the above equation (4.14) S_{corr} is the corrected scatter counts as described in equation 4.13, N is the systematic experimental error and k is a constant that is found using the calibration graph.

The trend line of the graph does not pass through zero but crosses the y-axis. This suggests that there is a systematic experimental error causing fewer counts to be recorded than expected. This is most likely due to a small amount of copper placed in the beam during the transmission measurements to protect the detector from the high photon flux. The geometry of the set-up was also changed between the scatter and transmission measurements. The detecor was moved further away. Due to the inverse square law this would mean that fewer counts would be recorded than expected. These two factors were not corrected for, as they can now be taken into account in this calculation.

As the composition of the calibration solutions are known the electron densities of these solutions can be calculated using the following formula

$$\rho_e = \rho N_A \sum \frac{Z_i}{A_i} \varpi_i \qquad A(4.15)$$

where ρ is the physical density of the material and Z/A is the ratio of atomic number to atomic weight for each element of mass fraction ω. Z/A vales are tabulated and were taken from Attix (1996). The graph in Figure 9 shows the theoretical electron densities plotted against the measured scattering coefficients

For this graph it can be seen that the two quantities correlate almost perfectly with a gradient equal to the Klein-Nishina cross section. This is what is expected as for high values of x the incoherent scattering factors become equal to Z. This agreement confirms the theoretical validity of the experiment.

Results

Figure 10 shows the results that were obtained from the scatter peak measurements.

On the chart in Figure 10 the median of each tissue type is shown (thick middle line). The interquartile range is contained within the box and the whiskers show the total range.

Analysis

Calculation of electron density values

The graph of Figure 8 gives a calibration equation to convert the number of counts in the scatter peak into the differential linear scatter coefficient μ_s. The equation given by the graph is

$$\mu_s = 1.737\times10^{-7}x + 7.919\times10^{-3} \qquad A(4.16)$$

where x is the corrected counts in the Compton peak.

These experimental scatter coefficients are then converted into electron densities using the calibration solution values. This conversion is shown by the trend line in the results graph (Figure 11).

The results are shown in the graph in Figure 11. On this graph the values of electron density for standard tissue compositions given in ICRU report 44 (ICRU, 1989) are also displayed. In this report three separate values are given for different tissue compositions. The elemental compositions of these six tissues have been given in the table below. It is worth noting that the values quoted in this report are for healthy tissues only, as there is no published data for malignant tissue growths.

Tissue	H	C	N	O	Other
Adipose #1	11.2	51.7	1.3	35.5	0.1 Na, 0.1 S, 0.1 Cl
Adipose #2	11.4	59.8	0.7	27.8	0.1 Na, 0.1 S, 0.1 Cl
Adipose #3	11.6	68.1	0.2	19.8	0.1 Na, 0.2 S, 0.1 Cl
Glandular #1	10.9	50.6	2.3	35.8	0.1 Na, 0.1 P, 0.1 S, 0.1 Cl
Glandular #2	10.6	33.2	3	52.7	0.1 Na, 0.1 P, 0.2 S, 0.1 Cl
Glandular #3	10.2	15.8	3.7	69.8	0.1 Na, 0.1 P, 0.2 S, 0.1 Cl

The elemental compositions (percentage by mass) of adult tissues (ICRU Report 44, 1989)

It is usually assumed that malignant tissue has approximately the same structure as healthy glandular tissue. This is because tumours are usually within fibrous tissue rather than growing in purely fatty (adipose) tissue.

The final results obtained are displayed in the table below.

Tissue	Electron density (e/cm^3)
Benign	$(3.362 \pm 0.141) \times 10^{23}$
Malignant	$(3.510 \pm 0.147) \times 10^{23}$
Adipose	$(3.312 \pm 0.139) \times 10^{23}$

Experimental values obtained for tissue electron densities

This Difference in measured electron density between tissue types can be used in a model, such as the one described in our co-pending UK patent application GB0328870.1, to determine the tissue type of samples for which the type is unknown. It therefore represents a potentially usefully diagnostic tool. As Compton scatter measurements can also be made in vivo, this approach also potentially lends itself to use as an in vivo, as well as in vitro, diagnostic approach.

Although the three tissue types used to exemplify the invention here are 'benign', 'malignant' and adipose', the approach can be applied to the determination of other tissue characteristics or other tissue analysis applications.

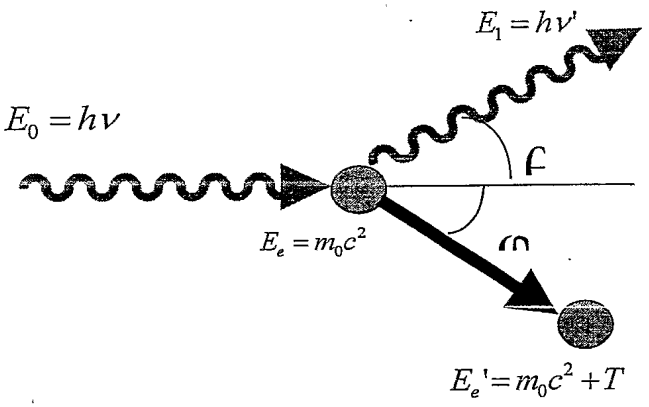

Figure 1

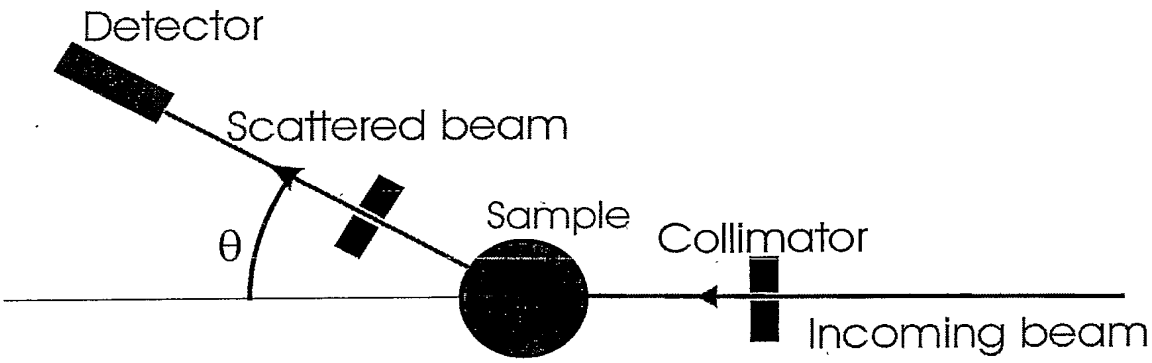

Figure 2

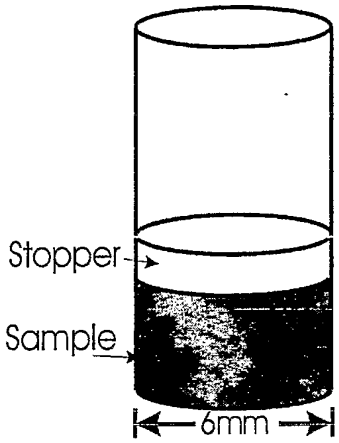

Figure 3

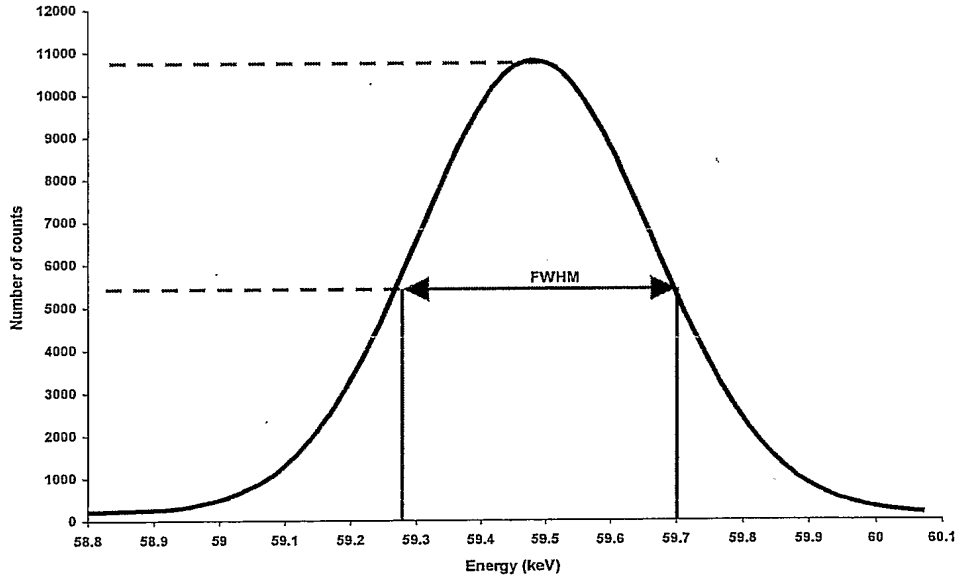

Figure 4

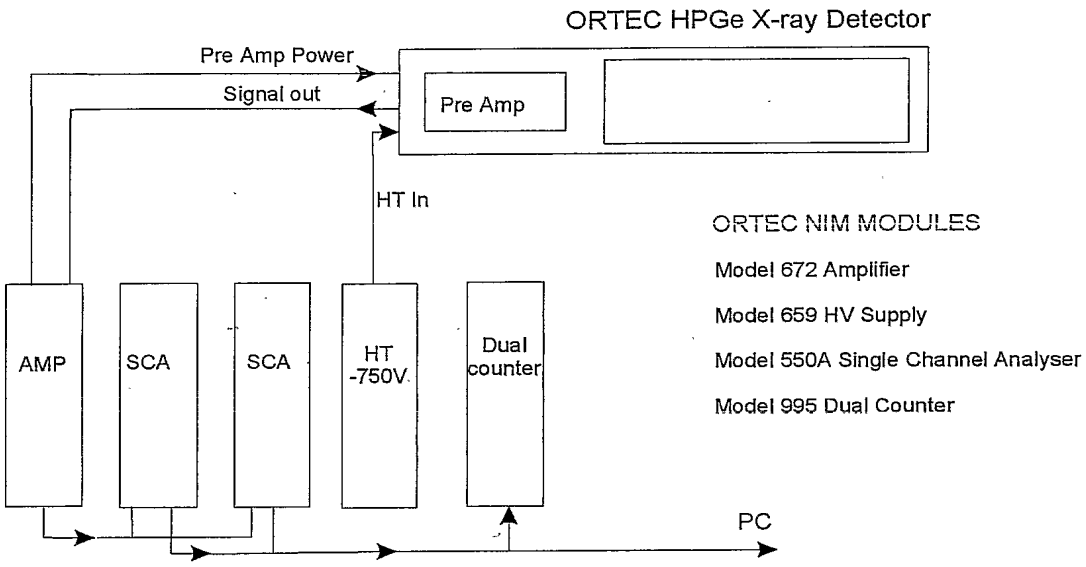

Figure 5

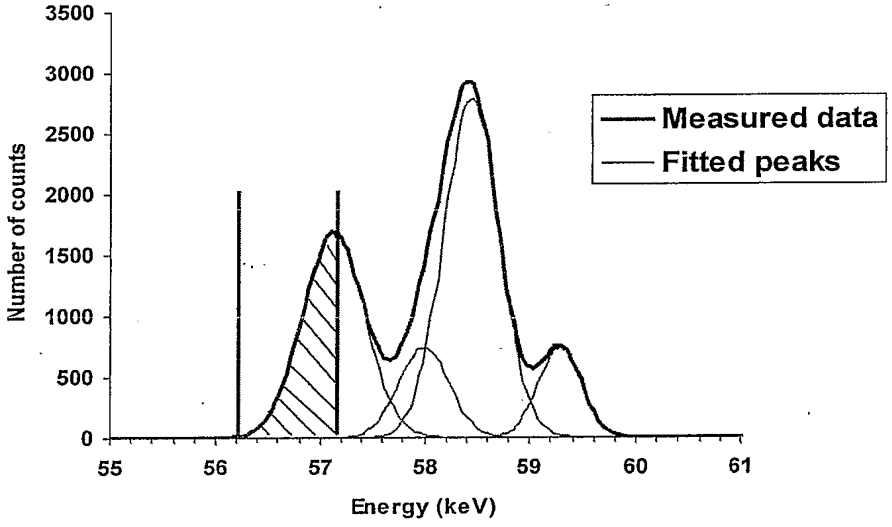

Figure 6

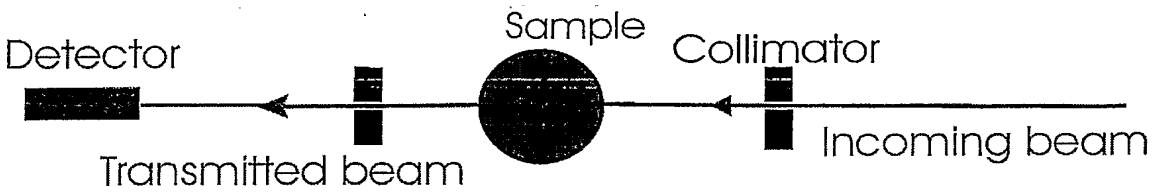

Figure 7

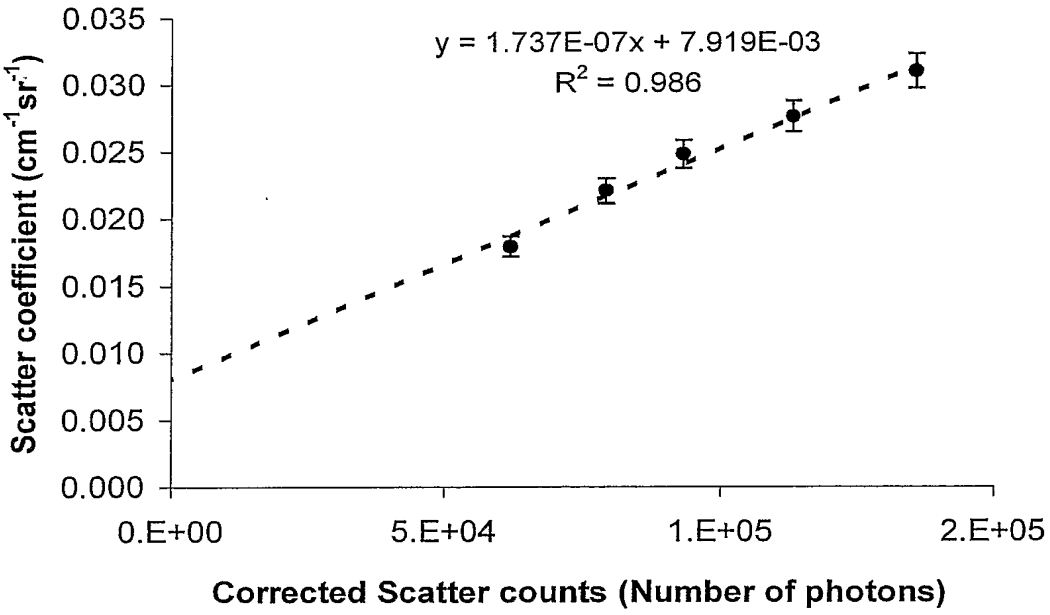

Figure 8

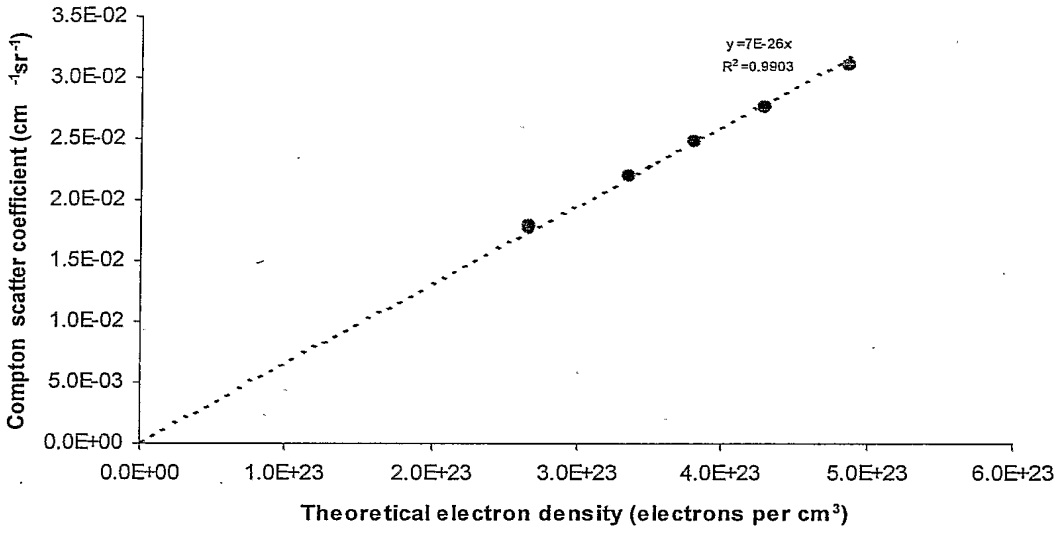

Figure 9

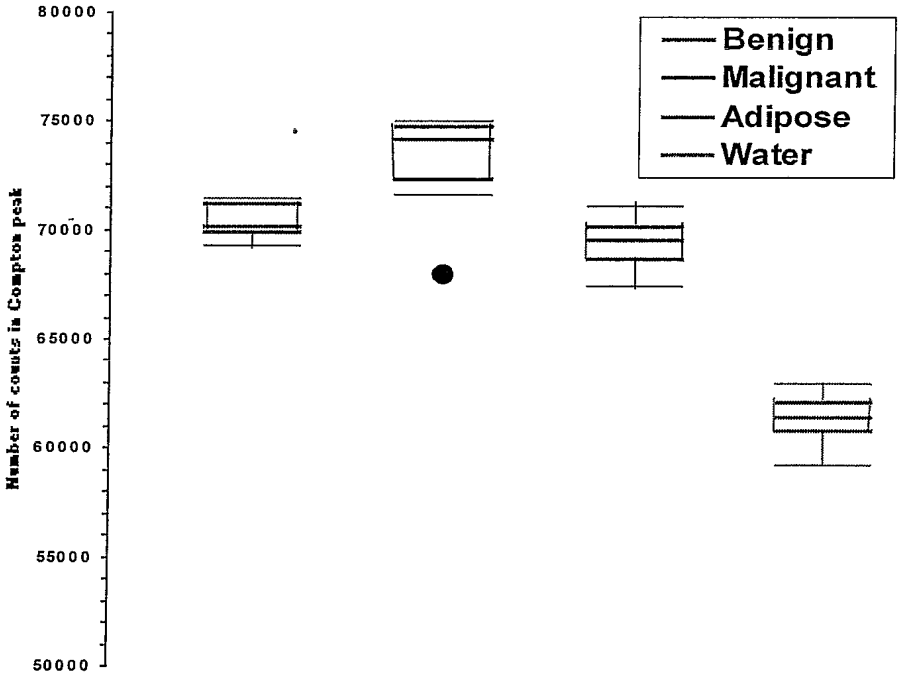

Figure 10

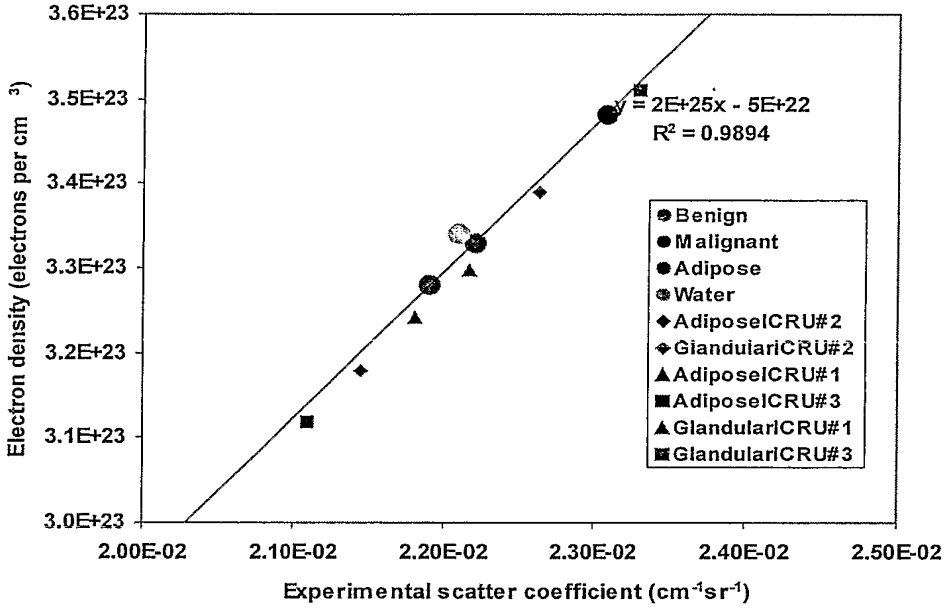
3.6E+23
3.5E+23
3.4E+23
3.3E+23
3.2E+23
3.1E+23
3.0E+23
Electron density (electrons per cm 3)
2.00E-02
2.10E-02
2.20E-02
2.30E-02
2.40E-02
2.50E-02
Experimental scatter coefficient (cm-1sr-1)
y = 2E+25x - 5E+22
R2 = 0.9894
Benign
Malignant
Adipose
Water
AdiposeICRU#2
GlandulariCRU#2
AdiposeICRU#1
AdiposeICRU#3
GlandulariCRU#1
GlandulariCRU#3

Figure 11

PCT/GB04/5185

DC